

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 September 2005 (22.09.2005)

PCT

(10) International Publication Number
WO 2005/087934 A3

(51) International Patent Classification⁷: **C12N 15/82**,
A01H 5/10, C12C 1/18

(21) International Application Number:
PCT/DK2005/000160

(22) International Filing Date: 9 March 2005 (09.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/800,200 11 March 2004 (11.03.2004) US

(71) Applicant (for all designated States except US): **CARLS-
BERG A/S** [DK/DK]; Valby Langgade 1, DK-2500 Valby
(DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BREDDAM, Klaus**
[DK/DK]; Agertoften 31, Østed, DK-4000 Roskilde (DK).
OLSEN, Ole [DK/DK]; Holmbladsgade 102 tv, DK-2300
Copenhagen S (DK). **SKADHAUGE, Birgitte** [DK/DK];
Lindehøjvej 31B, DK-3460 Birkerød (DK). **LOK, Finn**
[DK/DK]; Kongshaven 15, DK-2500 Valby (DK). **KNUD-
SEN, Søren** [DK/DK]; Bakkesvinget 18, DK-2760 Måløv
(DK). **BECH, Lene, Mølskov** [DK/DK]; Liljehaven 83,
DK-2765 Smørum (DK).

(74) Agent: **HØIBERG A/S**; St. Kongensgade 59A,
DK-1264 Copenhagen K (DK).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU,
ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted
a patent (Rule 4.17(ii)) for the following designations AE,
AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ,
CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE,
EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM,
PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA,
ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ,
NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM,
AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT,
BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR,
HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
2 February 2006

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: **BARLEY FOR PRODUCTION OF FLAVOR-STABLE BEVERAGE**

(57) Abstract: According to the invention, there is provided null-LOX-1 barley and plant products produced thereof, such as malt manufactured by using barley kernels defective in synthesis of the fatty acid-converting enzyme lipoxygenase-1. Said enzyme accounts for the principal activity related to conversion of linoleic acid into 9-hydroperoxy octadecadienoic acid, a lipoxygenase pathway metabolite, which-through further enzymatic or spontaneous reactions-may lead to the appearance of trans-2-nonenal. The invention enables brewers to produce a beer devoid of detectable trans-2-nonenal-specific off flavors, even after prolonged storage of the beverage.



WO 2005/087934 A3

INTERNATIONAL SEARCH REPORT

Int lional Application No
PCT/DK2005/000160

A. CLASSIFICATION OF SUBJECT MATTER
C12N15/82 A01H5/10 C12C1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A01H C12C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, COMPENDEX, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/053720 A (CARLSBERG RESEARCH LABORATORY; HEINEKEN TECHNICAL SERVICES BV; BRASSER) 11 July 2002 (2002-07-11) the whole document -----	1-8, 12-34, 39-43
X	WO 02/053721 A (CARLSBERG RESEARCH LABORATORY; HEINEKEN TECHNICAL SERVICES BV; BRASSER) 11 July 2002 (2002-07-11) the whole document -----	1-8, 12-31, 39-43, 46-51
P, X	WO 2004/085652 A (SAPPORO BREWERIES LIMITED; HIROTA, NAOHIKO; KANEKO, TAKAFUMI; KURODA,) 7 October 2004 (2004-10-07) the whole document ----- -/--	24-31, 39, 40

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

19 October 2005

Date of mailing of the international search report

21. 12. 2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Koch, J

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/167544 A1 (DOUMA ANNA CHRISTINA ET AL) 4 September 2003 (2003-09-04) claims 1,24 -----	32-34
X	KURODA H ET AL: "Characterization of factors that transform linoleic acid into di- and trihydroxyoctadecenoic acids in mash" JOURNAL OF BIOSCIENCE AND BIOENGINEERING, ELSEVIER, AMSTERDAM,, NL, vol. 93, no. 1, 2002, pages 73-77, XP002980834 ISSN: 1389-1723 abstract; table 1 -----	32-34
X	KOBAYASHI ET AL.: "Behavior of Mono-, Di-, and Trihydroxyoctadecenoic Acids during Mashing and Methods of Controlling Their Production" JOURNAL OF BIOSCIENCE AND BIOENGINEERING, vol. 90, no. 1, 2000, pages 69-73, XP002347966 figure 1; table 1 -----	32-34
A	RUTGERSSON A ET AL: "OPTIMIZATION OF TEMPERATURE, TIME, AND LACTIC ACID CONCENTRATION TO INACTIVATE LIPOXYGENASE AND LIPASE AND PRESERVE PHYTASE ACTIVITY IN BARLEY (CV. BLENHEIM) DURING SOAKING" CEREAL CHEMISTRY, AMERICAN ASSOCIATION OF CEREAL CHEMISTS. MINNEAPOLIS, US, vol. 74, no. 6, 1997, pages 727-732, XP000867116 ISSN: 0009-0352 page 727, right-hand column, paragraphs 1,2 abstract -----	32-34
A	DROST B W ET AL: "FLAVOR STABILITY" JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS, AMERICAN SOCIETY OF BREWING CHEMISTS, ST PAUL, MN, US, vol. 48, no. 4, 1990, pages 124-131, XP000926610 ISSN: 0361-0470 cited in the application the whole document -----	32-34
A	KURODA ET AL.: "Enzymes that Transform Linoleic Acid into Di- and Trihydroxyoctadecenoic Acids in Malt" MASTER BREWERS ASSOCIATION OF THE AMERICAS, vol. 40, no. 1, 2003, pages 11-16, XP008053559 the whole document -----	32-34

INTERNATIONAL SEARCH REPORT

national application No.
PCT/DK2005/000160

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-5, 12-31, 39-43, 46-51 (all partially); 6-8, 32-34 (all completely)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5,12-31,39-43,46-51 all partially; 6-8 all completely

Barley plant wherein the gene encoding LOX-1 of said plant comprises a nonsense codon corresponding to base nos. 3572-3574 of SEQ ID NO:2, methods and products based on said plant.

2. claims: 1-5,12-31,39-43,46-51 all partially; 9-11 all completely

Barley plant wherein the gene encoding LOX-1 of said plant comprises a splice site mutation said mutation corresponding to base no. 2311 of SEQ ID NO:6, methods and products based on said plant.

3. claims: 32-34 all completely

Beverage having stable organoleptic properties with a ratio of 9,12,13-trihydroxyoctadecenoic acid to 9,10,13-trihydroxyoctadecenoic acid of at the most 1.8

4. claims: 35-38 all completely

Beverage having stable organoleptic properties that comprises at the most 0.05 ppb free trans-2-nonenal (T2N) after incubation at 37 degrees C for 4 weeks in the presence of 4 to 6ppm sulfite

5. claim: 44 completely

A method of modulating levels of a protein to obtain a barley plant with less than 5% LOX-1 activity of a wild type barley plant by reducing gene expression of said protein by antisense or cosuppression silencing, or RNA interference

6. claim: 45 completely

A method of preparing a barley plant with less than 5% LOX-1 activity of a wild type barley plant by reducing gene expression of LOX-1 antisense or cosuppression silencing, or RNA interference.

7. claim: 52 completely

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method of producing a barley plant comprising less than 5% of the LOX-1 activity of a wild type barley plant by mutagenizing barley plants, kernels, embryos, cells, or tissues breeding said barley plants etc. for at least 2 generations, selecting and obtaining plants with desired characteristic.

8. claim: 53 completely

A method of producing a barley plant comprising less than 5% of the LOX-1 activity of a wild type barley plant by mutagenizing barley plants, kernels, or embryos, and optionally breeding said plants etc., and determining the presence of a mutation in the barley LOX-1 gene encoding a polypeptide form of LOX-1 comprising less than 700 contiguous amino acids of the sequence in SEQ ID NO:3

9. claim: 54 and 55 both completely

A method of reducing the activity of barley LOX comprising incubating a barley plant with a gallate type LOX inhibitor

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK2005/000160

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02053720	A	11-07-2002	NONE	
WO 02053721	A	11-07-2002	BG 107971 A CA 2433250 A1 HU 0401290 A2 SK 9372003 A3	30-09-2004 11-07-2002 28-09-2004 04-05-2004
WO 2004085652	A	07-10-2004	AU 2004223568 A1 JP 2004290024 A	07-10-2004 21-10-2004
US 2003167544	A1	04-09-2003	BR 0116579 A CN 1487995 A CZ 20031872 A3 EE 200300257 A EP 1346030 A1 JP 2004522434 T NZ 527171 A PL 363480 A1 ZA 200305786 A	20-04-2004 07-04-2004 12-11-2003 15-10-2003 24-09-2003 29-07-2004 29-07-2005 15-11-2004 29-12-2004